10/551603

JC20 Rec'd PCT/PTO 3 0 SEP 2005) REPLACEMENT SHEET

CLAIMS

WE CLAIM:

- 1. A human pluripotent embryonic stem cell culture, wherein the cells of the culture do not express SSEA1, express SSEA3, SSEA4, Oct4, Tra-1-60, Tra-1-80, and express nestin substantially uniformly.
- 2. The cell culture of Claim 1, wherein the cell culture was dissociated to an essentially single cell culture.
- 3. The cell culture of Claim 2, wherein a majority of the cells have an abnormal karyotype.
- 4. The cell culture of Claim 3, wherein the abnormal karyotype comprises a trisomy of at least one autosomal chromosome.
- 5. The cell culture of Claim 4, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
- 6. The cell culture of Claim 5, wherein the autosomal chromosome is chromosome 12 or 17.
- 7. The cell culture of Claim 3, wherein the abnormal karyotype comprises a trisomy of more than one autosomal chromosome.
- 8. The cell culture of Claim 7, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
- 9. The cell culture of Claim 8, wherein the autosomal chromosome is chromosome 12 or 17.
- 10. A method of culturing a human pluripotent embryonic stem cell comprising,
 - a) selecting a human pluripotent cell using an anti-SSEA4 antibody; and
 - b) maintaining a culture of the cell by passaging the cell using a protease treatment, wherein the cells of the culture do not express SSEA1, express SSEA3, SSEA4, Oct4, Tra-1-60, Tra-1-80, and express nestin substantially uniformly.
- 11. The method of Claim 10, wherein the protease treatment comprises the sequential use of Collagenase and trypsin.

- 12. The method of Claim 10, wherein the cell is maintained by using a protease treatment for at least 13 passages.
- 13. The method of Claim 10, wherein a majority of the cells of the culture have an abnormal karyotype.
- 14. The cell culture of Claim 13, wherein the abnormal karyotype comprises a trisomy of at least one autosomal chromosome.
- 15. The cell culture of Claim 14, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
- 16. The cell culture of Claim 13, wherein the abnormal karyotype comprises a trisomy of more than one autosomal chromosome.
- 17. The cell culture of Claim 16, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
- 18. The method of Claim 11, wherein Collagenase is used at a concentration of approximately 1 mg/ml for approximately 5 minutes, and wherein trypsin is used at a concentration of approximately 0.05% for approximately 30 seconds.
- 19. A method of providing a human cell culture enriched in neural cells, comprising forming an embryoid body comprising the human pluripotent embryonic stem cell of Claim 10.
- 20. The method of Claim 19, wherein the embryoid body is formed by culturing the cell with an essentially serum free medium.
- 21. The method of Claim 20, wherein the essentially serum free medium is a MEDII conditioned medium.
- 22. The method of Claim 21, wherein the MEDII conditioned medium is a Hep G2 conditioned medium.
- 23. The method of Claim 21, wherein the MEDII conditioned medium comprises one or more proline residues or a polypeptide containing proline residues.
- 24. The method of Claim 23, wherein the MEDII conditioned medium comprises proline at a concentration of approximately 50 μM.
- 25. The method of Claim 19, wherein the embryoid body is formed by culturing the cell with a minimal medium.
- 26. The method of Claim 25, wherein the minimal medium is essentially proline free.

- 27. The method of Claim 25, wherein the minimal medium comprises one or more proline residues, or a polypeptide containing proline residues.
- 28. The method of Claim 0, wherein the minimal medium comprises proline at a concentration from approximately 50 µM to approximately 250 µM.
- 29. The method of Claim 25, wherein the minimal medium is essentially FGF free.
- 30. The method of Claim 25, wherein the minimal medium is essentially MEDII free.
- 31. A human pluripotent cell produced by the method of Claim 10.
- 32. A human cell culture enriched in neural cells, produced by any the method of any one of Claims 19-30.
- 33. The human cell culture of Claim 32, wherein greater than approximately 80% of the human cell culture comprises neural cells.
- 34. The human cell culture of Claim 33, wherein greater than approximately 90% of the neural cells express tyrosine hydroxylase.
- 35. A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the human cell culture enriched in neural cells of Claim 32.
- 36. The method of Claim 35, wherein the neural disease is Parkinson's disease.
- 37. A method of culturing a human pluripotent embryonic stem cell comprising,
 - a) providing a human pluripotent embryonic stem cell culture;
 - b) passaging the cell culture using a protease treatment to thereby disperse the cell to an essentially single cell culture; and
 - c) culturing the essentially single cell culture in the presence of a feeder cell, a conditioned medium, or a minimal medium
 - to thereby culture the human pluripotent embryonic stem cell.
- 38. The method of Claim 37, wherein the protease treatment comprises the sequential use of Collagenase and trypsin.
- 39. The method of Claim 38, wherein Collagenase is used at a concentration of approximately 1 mg/ml for approximately 5 minutes, and wherein trypsin is used at a concentration of approximately 0.05% for approximately 30 seconds.
- 40. The method of Claim 37, wherein the feeder cell is a freshly plated feeder cell.

- 41. The method of Claim 40, wherein the feeder cell is a mouse embryonic fibroblast.
- 42. The method of Claim 40, wherein the feeder cell has been plated for less than 10 hours.
- 43. The method of Claim 40, wherein the feeder cell has been plated for less than 6 hours.
- 44. The method of Claim 40, wherein the feeder cell has been plated for less than 2 hours.
- A human pluripotent embryonic stem cell culture produced by the method of Claim 37, wherein the cells of the culture do not express SSEA1, express SSEA3, SSEA4, Oct4, Tra-1-60, Tra-1-80, and express nestin substantially uniformly.
- 46. The human pluripotent cell embryonic stem culture of Claim 45, wherein a majority of the cells of the culture have an abnormal karyotype.
- 47. The human pluripotent embryonic stem cell culture of Claim 46, wherein the abnormal karyotype comprises a trisomy of at least one autosomal chromosome.
- 48. The human pluripotent embryonic stem cell culture of Claim 47, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
- 49. The human pluripotent embryonic stem cell culture of Claim 46, wherein the abnormal karyotype comprises a trisomy of more than one autosomal chromosome.
- 50. The human pluripotent embryonic stem cell culture of Claim 49, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
- 51. A method of producing a human pluripotent embryonic stem cell culture enriched in neural cells comprising,
 - a) providing a human pluripotent embryonic stem cell culture;
 - b) passaging the cell culture using a protease treatment to thereby disperse the cell culture to an essentially single cell culture;
 - c) culturing the essentially single cell culture in the presence of a feeder cell, a conditioned medium, or a minimal medium; and

d) forming an embryoid body comprising the essentially single cell culture by culturing the cell culture with an essentially serum free medium,

to thereby produce the human cell culture enriched in neural cells.

- 52. The method of Claim 51, wherein protease treatment comprises the sequential use of Collagenase and trypsin.
- 53. The method of Claim 52, wherein Collagenase is used at a concentration of approximately 1 mg/ml for approximately 5 minutes, and wherein trypsin is used at a concentration of approximately 0.05% for approximately 30 seconds.
- 54. The method of Claim 51, wherein the essentially serum free medium is a MEDII conditioned medium.
- 55. The method of Claim 54, wherein the MEDII conditioned medium is a Hep G2 conditioned medium.
- 56. The method of Claim 54, wherein the MEDII conditioned medium comprises one or more proline residues or a polypeptide containing proline residues.
- 57. The method of Claim 56, wherein the MEDII conditioned medium comprises proline at a concentration of approximately 50 μM.
- 58. The method of Claim 51, wherein the feeder cell is a freshly plated feeder cell.
- 59. The method of Claim 58, wherein the feeder cell is a mouse embryonic fibroblast.
- 60. The method of Claim 58, wherein the feeder cell has been plated for less than 10 hours.
- 61. The method of Claim 58, wherein the feeder cell has been plated for less than 6 hours.
- 62. The method of Claim 58, wherein the feeder cell has been plated for less than 2 hours.
- 63. The method of Claim 51, wherein the minimal medium comprises one or more proline residues, or a polypeptide containing proline residues.
- 64. The method of Claim 63, wherein the minimal medium comprises proline at a concentration from approximately 50 μM to approximately 250 μM.
- 65. The method of Claim 51, wherein the minimal medium is essentially proline free.

- 66. The method of Claim 51, wherein the minimal medium is essentially FGF free.
- 67. The method of Claim 51, wherein the minimal medium is essentially MEDII free.
- 68. A human cell culture enriched in neural cells produced by the method of Claim 51.
- 69. A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the neural cell of Claim 68.
- 70. The method of Claim 69, wherein the neural disease is Parkinson's disease.
- 71. The human cell culture of Claim 68, wherein greater than approximately 80% of the human cell culture comprises neural cells.
- 72. The human cell culture of Claim 71, wherein greater than approximately 90% of the neural cells express tyrosine hydroxylase.
- 73. A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the human cell culture enriched in neural cells of Claim 68.
- 74. The method of Claim 73, wherein the neural disease is Parkinson's disease.